ABSTRACT

With UHT-sterilized milk as a model system, combinations of polyphosphate (.5 and 1.0%) and NaCl (.5 and 4.5%) were studied to determine their effects on the growth kinetics of *Listeria monocytogenes* Scott A and *Staphylococcus aureus* 196E. The milk was inoculated with 10^3 to 10^4 cfu/ml of either *L. monocytogenes* or *S. aureus* and incubated under aerobic conditions at 12, 19, 28, or 37°C. The addition of polyphosphate did not significantly inhibit the growth of either microbe at the temperatures studied, but the addition of NaCl or a combination of salts significantly inhibited growth. The addition of .5 or 1.0% polyphosphate alone to dairy products is not likely to affect substantially the growth of *S. aureus* or *L. monocytogenes*.

(**Key words**: sodium hexametaphosphate, ultra-high temperature milk, *Listeria monocytogenes*, *Staphylococcus aureus*)

Abbreviation key: **BHI** = brain-heart infusion, **SHMP** = sodium hexametaphosphate.

INTRODUCTION

Polyphosphates are used in a variety of food products as emulsifiers and moisture-binding agents (2, 4, 13). Pasteurized processed cheese food and spread are among the products containing polyphosphate. Polyphosphates inhibit various microorganisms in meat and meat products, depending on the type of phosphates used and the sensitivity of the microorganism (8, 13, 14). The addition of .5% polyphosphate to the brain-heart infusion (**BHI**) model system decreased the growth of *Listeria monocytogenes* and *Staphylococcus aureus* (5, 6, 7). The inhibitory effect of polyphosphate on *L. monocytogenes* was enhanced by the addition of 2.0% NaCl (15). However, no information exists on the antimicrobial effect of polyphosphates in the process cheese food or a milk model system.

The purpose of this investigation was to acquire data on growth kinetics on the effect of sodium hexametaphosphate (**SHMP**) in combination with low and high amounts of NaCl for *L. monocytogenes* and *S. aureus*, using UHT milk as a model for dairy products.

MATERIALS AND METHODS

Bacterial Strains and Media

Listeria monocytogenes Scott A and *S. aureus* 196E were used throughout this study. The inoculum was prepared by individual culture of the microorganisms for 18 to 24 h at 37°C in BHI broth (Difco, Inc., Detroit, MI) for *S. aureus* and in BHI broth supplemented with .3% glucose for *L. monocytogenes*. The overnight cultures were diluted to approximately 10^5 cfu/ml. All dilutions were made with sterile .1% peptone and water (Difco, Inc.). The BHI agar, tryptic soy agar, Baird-Parker agar with egg yolk-tellurite enrichment, and Vogel-Johnson agar were obtained from Difco, Inc.

Test Salts

The SHMP (Hexaphos®, a polyphosphate with 13 phosphate groups) was from FMC

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[1]Reference to a brand or firm name does not constitute an endorsement by the US Department of Agriculture over others of a similar nature not mentioned.

TABLE 1. Equations for Gompertz parameters and derived values for kinetics

Gompertz equation

$L(t) = A + C \exp(-\exp(-B(t - M)))$

where

$L(t)$ = $\log_{10}$ count of bacteria [log(cfu/ml)] at time (hours).
A = initial count of bacteria at start of growth study [log(cfu/ml)].
C = amount of bacterial growth [log(cfu/ml)] (final count at end of growth – initial count).
B = relative growth rate [log(cfu/ml)/h].
M = time (hours) for bacteria to reach stationary phase (cessation of growth – bacteria counts remain constant for a time).

Derived growth kinetics equations

Exponential growth rate is the time that cells divide at a constant rate = BC/2.71828 [log(cfu/ml)/h].
Generation time is the time that cells take to double in number = (log (2))2.71828/BC [h].
Lag phase duration is the time that cells take before they start to grow at a constant rate = M – (1/B) [h].
Maximum population density is the final count at the end of growth study = A + C[log(cfu/ml].

Corp (Philadelphia, PA). The SHMP solutions of 10 and 12% were prepared and added to the milk to obtain final concentrations of .5 and 1.0%, respectively. The solutions of NaCl (Mallinckrodt, Paris, KY) of 25 and 22.5% were prepared and added to the milk to obtain final concentrations of .5 and 4.5%, respectively. The test compounds were sterilized by filtration (.22 μm; Nalgene, Rochester, NY) before addition to the sterile milk to obtain the desired concentrations.

Culture Techniques

Homogenized UHT whole milk (Dairymen Inc., Savannah, GA) and the filtered, sterilized SHMP and NaCl solutions were added to 150-ml Erlenmeyer flasks to a total volume of 50 ml. Duplicate flasks were inoculated with .5 ml of the inoculum to achieve the initial 10^3 cfu/ml. All flasks were then incubated on a rotary shaker (100 rpm) at 12, 19, 28, or 37°C in accordance to the established procedure by Buchanan et al (1).

At appropriate intervals (0, 4, 6, 8, 24, 30, 48 h, etc., until logarithmic growth reached a plateau), samples were withdrawn, pH was measured, and aliquots were diluted and surface plated using a spiral plater (Spiral Systems™ Inc., Cincinnati, OH). Counts that had been enumerated on tryptic soy agar were compared with those enumerated on Baird-Parker or modified Vogel Johnson agar (11) and were found to be the same. For the remainder of the studies, tryptic soy agar was used as plating medium. The tryptic soy and Baird-Parker agar plates were incubated for 24 h at 37°C and then counted. The modified Vogel Johnson agar plates were incubated for 48 h at 37°C.

At the end of the logarithmic growth, samples of cultures incubated at 28°C were fixed in 1% glutaraldehyde. Cells were examined using a light microscope (Olympus BH-2; Tokyo, Japan) to determine the effect of NaCl and SHMP on cell size and characteristics.

Calculation of Growth Parameters

The Gompertz equation (3) in conjunction with ABACUS (USDA, ERRC, Philadelphia, PA), a nonlinear regression program employing a Gauss-Newton iteration procedure, was used to generate growth curves (Table 1). Gompertz A and C parameters were fixed using observed population density values. The Gompertz equation parameters (A, B, C, and M) were used to calculate exponential growth rates, generation times, lag times, and maximum cell population density.

Statistical Analysis

An ANOVA was performed to determine the significance of the effect of the addition of polyphosphates on the growth rate and generation times (10).

RESULTS AND DISCUSSION

The UHT-sterilized milk proved to be a very convenient model system for studying the

growth of foodborne pathogens in dairy products. *Listeria monocytogenes* and *S. aureus* grew in all SHMP and NaCl combinations at 19, 28, and 37°C. *Listeria monocytogenes* grew at 12°C, but *S. aureus* did not grow within 96 h under any test conditions. Generation times were inversely related to temperature. The generation times for *L. monocytogenes* grown in unsupplemented UHT-sterilized milk were 4.7, 1.7, 1.0, and .9 h at 12, 19, 28, and 37°C, respectively (Table 2). This generation time is somewhat faster than the average generation times reported by Rosenow and Marth (9) for the growth of *L. monocytogenes* in autoclaved whole milk. They (9) observed generation times of 5.8, 1.9, and .7 h at 13, 21, and 35°C, respectively. For *S. aureus*, generation times in the UHT-sterilized milk were 2.5, .4 and .3 h at 19, 28, and 37°C, respectively (Table 3). Stadhouders

TABLE 2. Growth kinetics of *Listeria monocytogenes* at 12, 19, 28, and 37°C in UHT milk.

Temperature	SHMP[1]	NaCl	Exponential growth rate		Lag phase duration		Generation time		Time for 10^3 increase	
(°C)	(%)		(h)							
			$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
12	0	0	.07	.01	1.6	.01	4.31	.37	51.5	3.0
	.5		.08	0	.5	1.50	3.63	.11	38.9	2.0
	1.0		.06	0	10.0	0	4.79	.20	68.3	3.0
	0	.5	.07	0	15.6	6.10	4.06	.18	58.5	5.7
	.5		.07	.01	25.8	.58	3.98	.17	69.0	8.0
	1.0		.08	0	25.0	3.80	3.93	.08	66.2	3.0
	0	4.5	.05	.01	26.5	14.10	6.55	.72	101.6	7.8
	.5		.04	0	46.2	5.20	6.73	.22	129.4	6.1
	1.0		.04	0	33.4	12.20	7.76	1.26	118.7	2.5
19	0	0	.11	.01	4.6	.02	1.68	.06	22.3	.6
	.5		.12	.01	4.9	.43	1.62	.01	22.0	.5
	1.0		.10	.01	5.6	2.30	1.65	.09	25.9	.4
	0	.5	.12	.01	5.4	1.68	1.61	.09	22.3	.8
	.5		.11	.01	4.4	.33	1.67	.02	21.7	.1
	1.0		.10	0	5.3	.08	1.85	.03	24.5	.3
	0	4.5	.08	0	4.0	1.50	2.03	.09	24.8	2.5
	.5		.10	.01	6.4	.15	1.98	.03	27.6	.1
	1.0		.09	0	7.1	.70	2.08	.01	29.3	.6
28	0	0	.18	.01	1.6	.10	1.00	.01	11.7	.1
	.5		.17	.10	1.7	.10	1.05	.05	12.7	.1
	1.0		.17	.01	2.3	.30	1.10	.01	13.8	.1
	0	.5	.22	.01	4.3	.01	.93	.01	14.4	.1
	.5		.21	.01	3.2	1.10	1.03	.01	14.5	.5
	1.0		.15	.01	3.3	.80	1.15	.05	15.2	.2
	0	4.5	.08	.01	.7	1.00	2.50	.30	26.7	.5
	.5		.09	.01	3.0	2.00	2.50	.10	31.1	.8
	1.0		.07	.01	4.3	4.20	1.80	.10	28.7	.5
37	0	0	.22	.02	1.2	.15	.94	.08	11.4	.6
	.5		.37	.04	2.8	.24	.63	.07	10.3	.4
	1.0		.32	.01	2.7	.07	.72	.02	11.4	.2
	0	.5	.30	.03	2.2	.40	.68	.05	9.5	.1
	.5		.57	.19	3.8	.72	.41	.14	8.2	.7
	1.0		.24	.06	2.0	.66	.89	.16	11.5	.9
	0	4.5	.13	0	4.4	1.01	1.33	0	18.3	1.0
	.5		.10	.01	2.9	1.30	1.74	.04	20.9	1.7
	1.0		.11	.01	5.1	.29	1.47	.13	20.0	1.8

[1]Sodium hexametaphosphate.

et al. (12), using HTST milk, reported that the generation times at 30°C varied from .3 to .6 h for several strains of *S. aureus*.

When the UHT-sterilized milk was used, the effects of added SHMP and NaCl on the growth kinetics were determined for *L. monocytogenes* (Table 2) and *S. aureus* (Table 3). Growth rate, duration of lag phase, generation time, and times for an increase in cell number of 10^3 at 19, 28, and 37°C were calculated. No activity against *S. aureus* 196E occurred when .5 or 1.0% SHMP was added to the UHT milk at 19, 28, and 37°C (Table 4), in contrast to the results of Jen and Shelef (6), who reported that the addition of .5% SHMP to BHI had a bactericidal effect on *S. aureus* 196E at 35°C.

Supplementation of UHT milk with .5 or 1.0% SHMP did not affect the growth rate or generation times of *L. monocytogenes* (Table 4). However, lag phase and the time for a 10^3 increase in population density increased for *L. monocytogenes* with the addition of 1.0% SHMP but was not significant (Table 4). Zaika and Kim (15) found that the addition of .5 or 1.0% SHMP to BHI (pH 6.0) decreased the exponential growth rate and increased generation and lag times for *L. monocytogenes* grown at 5, 10, 19, and 28°C. Both research groups (6, 15) postulated that added SHMP in BHI chelated cations necessary for the growth of the microorganisms. Jen and Shelef (6) found that inhibitory activity of SHMP was partially eliminated by the addition of Ca (.25 to 1 m*M*) and completely eliminated by Mg (.25 to .35 m*M*). The high concentrations of Ca (30 m*M*) and Mg (5 m*M*) in milk probably eliminated any potential inhibitory effect by the SHMP. Therefore, care must be exercised in extrapolation of antimicrobial effects observed in microbiological media to food systems.

TABLE 3. Growth kinetics of *Staphylococcus aureus* at 19, 28, and 37°C in UHT milk.

Temperature	SHMP[1]	NaCl	Exponential growth rate		Lag phase duration		Generation time		Time for 10^3 increase	
(°C)	(%)		(h)							
			$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
19	0	0	.08	.01	14.0	5.5	2.44	.01	40.3	4.1
	.5		.09	.01	13.1	3.5	2.14	.15	38.3	2.6
	1.0		.08	.01	14.2	1.9	2.27	.07	38.6	.1
	0	.5	.11	.01	6.9	.3	1.82	.06	26.4	1.0
	.5		.11	.01	6.0	2.1	1.93	.11	27.5	.9
	1.0		.09	.01	8.1	.8	2.09	.28	30.3	2.3
	0	4.5	.10	.01	4.9	.1	2.08	.15	27.1	1.4
	.5		.08	.01	3.6	.1	2.25	.10	27.0	.9
	1.0		.05	.01	1.4	.1	3.49	.04	37.1	.4
28	0	0	.38	.01	2.8	.2	.39	.02	7.0	.1
	.5		.38	.03	2.9	.1	.40	.02	6.9	.2
	1.0		.36	.06	2.8	.1	.43	.02	7.1	.3
	0	.5	.33	.01	2.2	.2	.55	.05	8.0	.3
	.5		.28	.01	2.1	.1	.60	.05	8.4	.2
	1.0		.33	.05	3.1	.6	.55	.05	9.0	.2
	0	4.5	.28	.05	4.0	.6	.65	.15	10.7	.7
	.5		.16	.01	2.9	.4	1.10	.10	14.2	.2
	1.0		.10	.01	2.1	.1	1.75	.15	20.2	1.4
37	0	0	.38	.01	1.8	.1	.31	.01	4.9	.1
	.5		.73	.04	3.8	.8	.16	.02	5.4	.5
	1.0		.53	.03	2.8	.1	.24	.01	5.2	.1
	0	.5	.32	.01	1.0	.1	.51	.01	6.2	.1
	.5		.36	.02	1.4	.1	.47	.04	6.2	.4
	1.0		.32	.04	1.4	.1	.53	.08	6.8	.4
	0	4.5	.45	0	2.8	.1	.38	.01	6.7	.1
	.5		.53	.03	3.4	.2	.35	.01	7.0	.1
	1.0		.46	.03	2.6	.1	.37	.04	6.4	.4

[1]Sodium hexametaphosphate.

The pH of the UHT milk was 6.5 to 6.6, which changed slightly, to pH 6.6 to 6.8, after the addition of SHMP. The pH of the UHT milk system remained the same during the growth of the *L. monocytogenes* but decreased to 5.2 ± .1 during the growth of the *S. aureus*. Jen and Shelef (6) also reported increased pH with the addition of SHMP to BHI; they found that inhibition by polyphosphates was dependent on pH and that inhibition increased at higher pH, >7.0. In the present study, the pH of the UHT milk system remained well below the 7.0 considered to be a factor for increasing inhibition by SHMP.

In the UHT milk system, the addition of .5 and 4.5% NaCl overall affected the growth kinetics of *S. aureus* and *L. monocytogenes*; these effects were statistically significant (Tables 4 and 5). Buchanan et al. (1) previously reported that the addition of 4.5% NaCl to the tryptose-phosphate broth did not have a major effect on growth kinetics of *L. monocytogenes*. Zaika and Kim (15) also reported that the addition of 2.0% NaCl did not have a major effect on the growth kinetics of *L. monocytogenes*. However, the addition of 2.0% NaCl enhanced the inhibitory effect of .5 and 1.0% SHMP in BHI, pH 6.0, at 28 and 19°C. They (15) reported increased generation times and lag phase durations for *L. monocytogenes*. In the current study, the reverse occurred for *L. monocytogenes* in UHT milk. The addition of SHMP alone was not significant (Table 4) as an inhibitor of *L. monocytogenes* but enhanced the inhibitory effect of NaCl, as evidenced by a decrease in growth rate with increased SHMP.

Microscopic examination of the cells in log growth at 28°C for all NaCl and SHMP combinations in the UHT milk model system showed normal cellular shapes. The cellular shape of *L. monocytogenes* grown in the presence of .5% polyphosphate in the BHI system has been reported (L. L. Zaika, P. Cooke, and T. Dobson, May 1991, personal communication). The cells grew in elongated shapes, and ultrastructure examination showed incomplete cell walls between the dividing cells. In the present

TABLE 4. Analysis of variance on growth kinetics of *Staphylococcus aureus* and *Listeria monocytogenes*.

Treatment[1]	df	MS			
		Growth rate	Lag phase duration	Generation time	Time for 1000 increase
		S. aureus			
Model					
Temp	2	4.06**	73.57	15.25**	2628.02**
SHMP	2	.07	7.99	.23	50.76*
Temp × SHMP	4	.19	2.65	.04	2.00
NaCl	3	.75*	38.29**	1.12**	66.13**
Temp × NaCl	3	.59	22.84*	.48**	85.08**
SHMP × NaCl	6	.12	10.18	.60**	28.81*
Temp × SHMP × NaCl	6	.08	3.88	.11	4.14
Error	27	.16	5.25	.07	10.66
Corrected total	53				
		L. monocytogenes			
Model					
Temp	3	.85**	1334.49**	64.83**	16,282.87**
SHMP	2	.03	92.18	.09	52.97
Temp × SHMP	6	.04	54.95	.09	42.37
NaCl	2	.75**	453.31**	19.80**	4765.32**
Temp × NaCl	6	.19*	365.81**	2.97**	1438.76**
SHMP × NaCl	4	.07	59.28	.36	55.80
Temp × SHMP × NaCl	12	.02	59.92*	.57	60.27
Error	42	.06	28.73	.42	33.56
Corrected total	77				

[1]SHMP = Sodium hexametaphosphate; Temp = temperature.

*$P < .05$.

**$P < .01$.

TABLE 5. Means of lag phase duration and generation time for temperature versus percentage of NaCl averaged over the amounts of sodium hexametaphosphate.

	Lag phase duration			Generation time		
Temperature	0% NaCl	.5% NaCl	4.5% NaCl	0% NaCl	.5% NaCl	4.5% NaCl
(°C)	*Listeria monocytogenes*					
12	3.55	22.1	35.3	3.87	3.99	7.01
19	5.00	5.02	5.80	1.65	1.70	2.03
28	1.84	4.33	−1.82	1.04	.79	2.84
37	2.22	2.35	4.12	.76	.48	1.51
SE	2.19			.26		
LSD	6.26			.75		
	Staphylococcus aureus					
19	10.10	8.85	3.29	2.39	1.59	2.61
28	4.33	2.43	2.97	.53	.58	1.16
37	1.24	2.94	2.85	.28	.50	.36
SE	.93			.11		
LSD	2.66			.32		

study, the *L. monocytogenes* cells were normal and rod-shaped with no evidence of elongation; the *S. aureus* cells were normal with some evidence of pairing, as is typical for this organism.

CONCLUSIONS

In the UHT-sterilized milk model system, the addition of SHMP (1%) alone did not have an effect on the growth kinetics of *S. aureus* and had only a minor effect on the growth kinetics of *L. monocytogenes*. Growth was inhibited for both pathogens when .5 and 4.5% NaCl alone were added to the UHT-sterilized milk. Inhibitory effects of NaCl apparently were enhanced when 1% SHMP was added to the milk model system.

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